# Inland Waters & Catchment Ecology

# Effects of hyper-saline conditions upon six estuarine fish species from the Coorong and Murray Mouth



## D. G. McNeil, S. Westergaard, K. J. M. Cheshire, C. J. Noell and Q. Ye

## July 2013

Government of South Australia

# Effects of hyper-saline conditions upon six estuarine fish species from the Coorong and Murray Mouth

## D. G. McNeil, S. Westergaard, K. J. M. Cheshire, C. J. Noell and Q. Ye

## July 2013

**South Australian Research and Development Institute**
SARDI Aquatic Sciences
2 Hamra Avenue
West Beach SA 5024

Telephone: (08) 8207 5400
Facsimile: (08) 8207 5406
http://www.sardi.sa.gov.au

## DISCLAIMER

Printed in Adelaide: September 2013

SARDI Publication No. F2009/000014-4
SARDI Research Report Series No. 700

Author(s):     D. G. McNeil, S. Westergaard, K. J. M. Cheshire, C. J. Noell and Q. Ye

Reviewer(s):    S. Madigan, M. Geddes, M. Steer, J. Tanner and R. Ward

Approved by:   X. Li
                 Science Leader - Aquaculture

Signed:

Date:             6 September 2013

Distribution:   FRDC, SAASC Library, University of Adelaide Library, Parliamentary Library, State Library and National Library

Circulation:    Public Domain

# Table of Contents

## List of Figures

# List of Tables

# Acknowledgements

# Executive Summary

This report investigates the tolerance thresholds of key Coorong fish species' juveniles to hyper-marine salinity and the relationship between thresholds and distribution of species and salinity levels in the field. These species were black bream (*Acanthopagrus butcheri*), yellow eye mullet (*Aldrichetta forsteri*), Tamar goby (*Afurcagobius tamarensis*), greenback flounder (*Rhombosolea tapirina*) and congolli (*Pseudaphritis urvillii*), mulloway (*Argyrosomus hololepidotus*) and smallmouthed hardyhead (*Atherinosoma microstoma*). Gradual acclimation style tolerance trials (increase in salinity at a rate of 2 ppt per day from an initial concentration of 35 ppt) were conducted within aquaria maintained at two different test temperatures; 14 ℃ (representative 'winter' temperature) and 23 ℃ (representative 'summer' temperature). Three replicate trials were conducted for each species. Experimental measurements were conducted daily to estimate lethal (number of mortalities and percent survival) and sub-lethal (behaviour measurements relating to stress, e.g. number of fish actively swimming, the presence of any disease or health conditions and maintenance of equilibrium and diminished feeding activity) thresholds to salinity. Results were then analysed using Probit analysis to give a species Lethal Concentration (LC) value. LC values were estimated for levels of salinity that were lethal for 10% ($LC_{10}$), 50% ($LC_{50}$) and 90% ($LC_{90}$) of test fish. Daily Feeding Response Scores (DFRS) and Stress Scores (SS) were also calculated from behavioural observations. Spatial and temporal abundance data for each of the selected species and field salinity data (parts per thousand, ppt) were adapted from Noell *et al.* (2012). Juveniles of all fish species trialled tolerated high salinity levels approximately twice the concentration of seawater. There was, however, a gradient of relative tolerance across species. Behavioural response measures during gradual salinity increase provided additional insight into the sub-lethal salinity impacts. The maximum field salinities at which species were caught divided species into two groups with species captured in salinities greater than 74 ppt and species captured in salinities less than 60 ppt. These field patterns suggest that the winter temperature tolerance thresholds for these species were closely related to their distribution throughout the Coorong during 2006 to 2008. A key outcome of the current study is the application of experimentally derived tolerance thresholds to the distribution of fish across a natural salinity gradient in the field. The results suggest that threshold values, in particular $LC_{10}$ and $LC_{50}$, can approximate the maximum salinity extent of field distribution of species with moderate accuracy.

# 1. Introduction

The allocation of water resources away from the environment have greatly reduced freshwater flows in the Murray-Darling Basin over the past fifty to a hundred years (Montagna *et al*., 2002; Flemer and Champ, 2006; Vorwerk *et al*., 2008). In south eastern South Australia this has been matched by the draining of vast wetland areas, critical for providing freshwater inflows to coastal wetlands (Pressey and Adam, 1995). The result has been a reduction in the delivery of freshwater inflows to the Coorong and Lower Lakes of the River Murray, both via the reduction of river flows and a reduction in groundwater and landscape seepage from the south east wetlands. This has led to a significant decline in ecosystem health (Boon, 2000; Walker, 2002; Webster, 2005; Noell *et al*., 2009).

The Murray Mouth has been dredged since 2001 to avoid closure caused by depleted flow volumes and accumulated sediments, however, the lack of freshwater input means the Murray Mouth and Coorong is now effectively a tidal marine inlet with a reverse estuarine structure. Salinity increases with distance from the mouth, generally ranging between saline in the Murray Mouth to hyper-saline in the northern and southern Lagoons (Noell *et al*., 2009). The resultant salinity gradient has important implications for the ecological health of the Coorong (e.g. Geddes and Butler, 1984; Geddes, 2005a; 2005b). With such a reduction in freshwater inputs, the estuarine structure of coastal lagoons like the Coorong deteriorates. If open to the sea, conditions are likely to become predominantly marine (Whitfield, 2005) but, if closed, conditions can rapidly become hyper-saline with salinity concentration reaching many times the level of seawater (Vega-Cendejas and Hernández de Santillana, 2004; Whitfield, 2005).

Estuaries and coastal lagoons play a significant role in providing spawning grounds, nursery areas and migratory pathways for fish (Pollard, 1971; Blaber *et al*., 1989; Potter *et al.*, 1990; Whitfield, 1999; Zampatti *et al*., 2010). Shifts away from estuarine to marine or hyper-saline conditions can impact upon the ecological stability of fish populations. With increasing salinity, key ecological processes such as spawning, recruitment, habitat use and/or food resource availability can be impacted (James *et al*., 2003). Under hyper-saline conditions, estuarine and even marine adapted fishes may become physiologically challenged as the requirement for osmotic homeostasis places increasing demand on metabolic processes (Boeuf and Payan, 2001; Hildebrand and Goslow, 2001).

In the Coorong and Murray Mouth, field based research has indicated a strong correlation between salinity levels and distribution of fish species and assemblage groups. This supports observations that salinity is a key factor driving the fragmentation of fish distributions in the Coorong and Lower Lakes region (Wedderburn *et al..* 2008). Recent tolerance data have shown that freshwater fishes from the River

Murray and Lower Lakes are under threat from rising salinity levels that may preclude the survival of sensitive larval life stages leading to recruitment failure for some species (Ye *et al.*, 2010). For the estuarine fish assemblage of the Coorong, however, tolerance values have not yet been assessed to allow comparisons of limiting physiological thresholds and ecological distributions of fish species in relation to salinity.

The aim of the current study is to identify the tolerance thresholds of key Coorong fish species to hyper-saline salinity levels and apply these threshold values to the distribution of species and salinity levels in the field. In particular, thresholds were identified for juvenile age classes which may be more sensitive to salinity impacts than adults (Hart *et al.*, 1991; Clunie *et al.*, 2002). In addition, tolerance values were assessed under average summer and winter temperature conditions to maximize the applicability of thresholds under fluctuating seasonal conditions. These values were then compared with the spatial and temporal distribution of salinity and fish species in the Coorong as presented in Noell *et al.* (2009) to determine the relationship between fish species tolerances, distribution and salinity levels.

The specific aims of the study were:

1. Determine the tolerance thresholds for key fish species (juveniles) from the Coorong and Murray Mouth.

2. Compare tolerance values under summer and winter temperature regimes to identify confounding impacts of season on tolerance.

3. Relate tolerance thresholds to the spatial and temporal distribution of fish and salinity levels throughout the Coorong.

4. Discuss the implications of salinity/fish assemblage relationships to the ecological management of the Coorong and Murray Mouth.

## 2.  Methods

**Target fish species**

Juveniles of black bream (*Acanthopagrus butcheri*), yellow eye mullet (*Aldrichetta forsteri*), Tamar goby (*Afurcagobius tamarensis*), greenback flounder (*Rhombosolea tapirina*) and congolli (*Pseudaphritis urvillii*) and mulloway (*Argyrosomus hololepidotus*) were selected for salinity tolerance trials (Table 1). Their tolerance to hyper-marine salinity and the relationship between thresholds and distribution of species along the Coorong salinity gradient in the field were investigated with an additional species, smallmouthed hardyhead (*Atherinosoma microstoma*), included.

**Table 1. Life history styles of target species adapted from Noell *et al.* (2009).**

| Common name | Species name | Life history* | Habitat use |
|---|---|---|---|
| Mulloway | *Argyrosomus hololepidotus* | Marine opportunist | Pelagic |
| Tamar goby | *Afurcagobius tamarensis* | Estuarine | Benthic |
| Black bream | *Acanthopagrus butcheri* | Estuarine | Pelagic |
| Greenback flounder | *Rhombosolea tapirina* | Estuarine and marine | Benthic |
| Yellow eye mullet | *Aldrichetta forsteri* | Marine opportunist | Pelagic |
| Congolli | *Pseudaphritis urvillii* | Catadromous | Benthic |
| Smallmouthed hardyhead | *Atherinosoma microstoma* | Estuarine | Pelagic |

* Potter and Hyndes (1994).

**Experimental setup: slow acclimation tolerance trials**

Juvenile fish (size ranges provided in Table 2) were collected from the Lower River Murray and Coorong, except for *Argyrosomus hololepidotus* juveniles which were hatchery stock. Fish were transferred into 2000 L aquaria under controlled environment conditions at the SARDI Aquatic Sciences Centre in West Beach, South Australia. All fish were quarantined and treated for potential disease as per Australian Pest and Veterinary Medicine Authority permit 8853. Test fish were acclimated in sea water within experimental aquaria for at least one week under experimental conditions prior to testing. During this period, any mortalities were replaced with fish from a holding aquarium subjected to identical conditions as experimental tanks.

Five fish were acclimated within each of three replicated 20 L experimental aquaria and at the onset of experimentation salinity was increased. Gradual acclimation style tolerance trials were conducted within aquaria maintained at two different test temperatures; 14 ºC (representative 'winter' temperature) and 23 ºC (representative 'summer' temperature). At the onset of experiments, test aquaria were subjected to a gradual increase in salinity at a rate of 2 ppt per day from an initial concentration of 35 ppt with the use of Ocean Nature® or Sea-Chem® salt mixed with filtered seawater. Daily measurements of key water quality parameters were recorded including dissolved oxygen, total dissolved solids (as a measure of salinity), pH and temperature.

Experimental measurements were then recorded including: number of mortalities, number of fish actively swimming, presence of any disease or health conditions and maintenance of equilibrium (i.e. maintaining balanced position in the water). An index of feeding response (Table 3) and an index of visual stress (Table 4) were also recorded daily. Finally, salinity was reset at the next incremental level within treatment tanks (i.e. 2 ppt higher than the previous day).

Throughout acclimation and trials, a light:dark ratio approximating 12:12 hours was used. Fish were fed daily. Initially during acclimation a varied diet was offered, which included frozen bloodworm, frozen brine shrimp (*Artemia* sp.), live brine shrimp, and 1 mm or 5/8 proton aquaculture pellet. Diet throughout the trials remained consistent but was dependent on species-specific acceptance of these foods observed during acclimation (Table 2). High levels of ammonia are known to be detrimental to fish survival and also may affect bio-filter performance. Thus, prior to the start of each trial, ammonia was tested in a sample of tanks to ensure levels were suitable.

**Table 2. Weight and length (mean ± SD) of test fish and nutrition supplied for each species in the 14 ºC and 24 °C trials.**

| Common name | Species | 14 °C | | 23 °C | | Food supplied pre-trial |
|---|---|---|---|---|---|---|
| | | Mean weight (g) | Mean total length (TL, mm) | Mean weight (g) | Mean total length (TL, mm) | |
| Mulloway | *Argyrosomus hololepidotus* | - | 45-75 | - | 45-75 | aquaculture pellet |
| Tamar goby | *Afurcagobius tamarensis* | 1.83 ± 1.42 | 63.9 ± 14.9 | 1.87 ± 1.65 | 58 ± 17.3 | bloodworm and *Artemia* |
| Black bream | *Acanthopagrus butcheri* | 0.89 ± 0.94 | 44.1 ± 13.1 | 0.75 ± 0.53 | 43.3 ± 10.3 | aquaculture pellet |
| Greenback flounder | *Rhombosolea tapirina* | 0.41 ± 1.35 | 35 ± 17 | 0.27 ± 0.61 | 30 ± 15 | bloodworm and *Artemia* |
| Yellow eye mullet | *Aldrichetta forsteri* | 0.80 ± 1.08 | 47.2 ± 16.8 | 1.02 ± 0.86 | 51.2 ± 8.4 | aquaculture pellet |
| Congolli | *Pseudaphritis urvillii* | 0.25 ± 0.21 | 39.0 ± 7.9 | 0.24 ± 0.11 | 40.6 ± 7.3 | bloodworm and *Artemia* |

## Behavioural observations

Daily assessment of fish behaviour via observation was performed prior to resetting of salinity. The assessment informed and complemented the measurement of lethal thresholds and provided insight into some of the potential sub-lethal thresholds that indicate physiological impact prior to lethality. Behaviour measurements relating to diminished feeding activity and stress were taken based on Daily Feeding Response Scores (DFRS) and Stress Scores (SS) (Table 3 and Table 4, respectively). These were made by the same researcher to minimise experimental variation in observational scoring.

**Table 3. Daily Feeding Response Score (DFRS)**

| DFRS | | Description |
| --- | --- | --- |
| 5 | High response | Feeding eagerly at surface, feed not reaching floor of tank. |
| 4 | Medium high response | Some feed reaching floor of tank, but consumed within 5mins. |
| 3 | Medium response | Most feed reaching floor of tank, but consumed within half an hour. |
| 2 | Medium low response | Feed reaching floor of tank, more than half consumed within half an hour. |
| 1 | Low response | Feed reaching floor of tank, less than half consumed within half an hour. |
| 0 | No response | Feed not consumed, little interest in feeding observed. |

**Table 4. Stress Score (SS)**

| SS | | Description |
| --- | --- | --- |
| 5 | High stress | Dark obvious banding increased opercula pumping and constant hiding. |
| 4 | Medium high stress | Dark obvious banding, slightly increased opercula pumping and hiding majority of time. |
| 3 | Medium stress | Some dark banding, often accompanied by hiding. |
| 2 | Medium low stress | Faint banding, some hiding from movement. |
| 1 | Low stress | No banding, some hiding from movement. |
| 0 | No stress | Little flight response, swimming at front of tank in anticipation of feeding. |

## Data analysis and threshold determination

The percent survival of test fish under gradually increasing salinity was measured daily for each trial. Results from the three replicate trials for each species were then analysed using Probit analysis to give a species Lethal Concentration (LC) value. LC values were estimated for levels of salinity that were lethal for 10% ($LC_{10}$), 50% ($LC_{50}$) and 90% ($LC_{90}$) of test fish. For *Atherinosoma microstoma* the $LC_{10}$, $LC_{50}$, and $LC_{90}$ values were estimated from data reported by Lui (1969). Probit analyses were carried out using SPSS Version 16.0 and StatsDirect Version 2.6.2. Salinity concentration (ppt) was $log_{10}$ transformed for analysis.

Spatial and temporal abundance data for each of the selected species and field salinity data (ppt) were adapted from Noell *et al.* (2012). Abundances of each species were overlaid as bubbles onto contour salinity graphs and presented through time with increasing distance within the Coorong from the Murray Mouth. $LC_{50}$ estimates at 14 °C and 23 °C were applied as a threshold of expected field distribution.

# 3.  Results

All species tested displayed tolerance to salinity conditions greater than seawater, with $LC_{50}$ estimates ranging between 54.4 ppt (*A. hololepidotus*) and 100.1 ppt (*P. urvillii*) (Figure 1). Tolerance estimates for *A. microstoma* measured by Lui (1969) were slightly higher than other species measured ($LC_{50}$ = 108 ppt), although were comparable with field observations for the species. Relative species tolerances were similar across species for both temperatures (as graphed from left to right − Figure 1) with somewhat lower tolerance values recorded at 23 °C than at 14 °C. Field salinity maxima for species presence ranged from approximately 60 ppt to over 130 ppt for *A. microstoma.* Species were rarely captured under salinity conditions higher than their laboratory derived $LC_{50}$ thresholds, although *A. microstoma* was caught under salinities well above their estimated threshold was calculated for both temperatures and *A. hololepidotus* was captured in the field at salinities higher than their estimated 23 °C $LC_{50}$ threshold. More detailed analysis of survival under increasing salinity, tolerance thresholds and spatio-temporal distributions of fish and salinity levels in the Coorong are presented individually for each target species in the following sections.
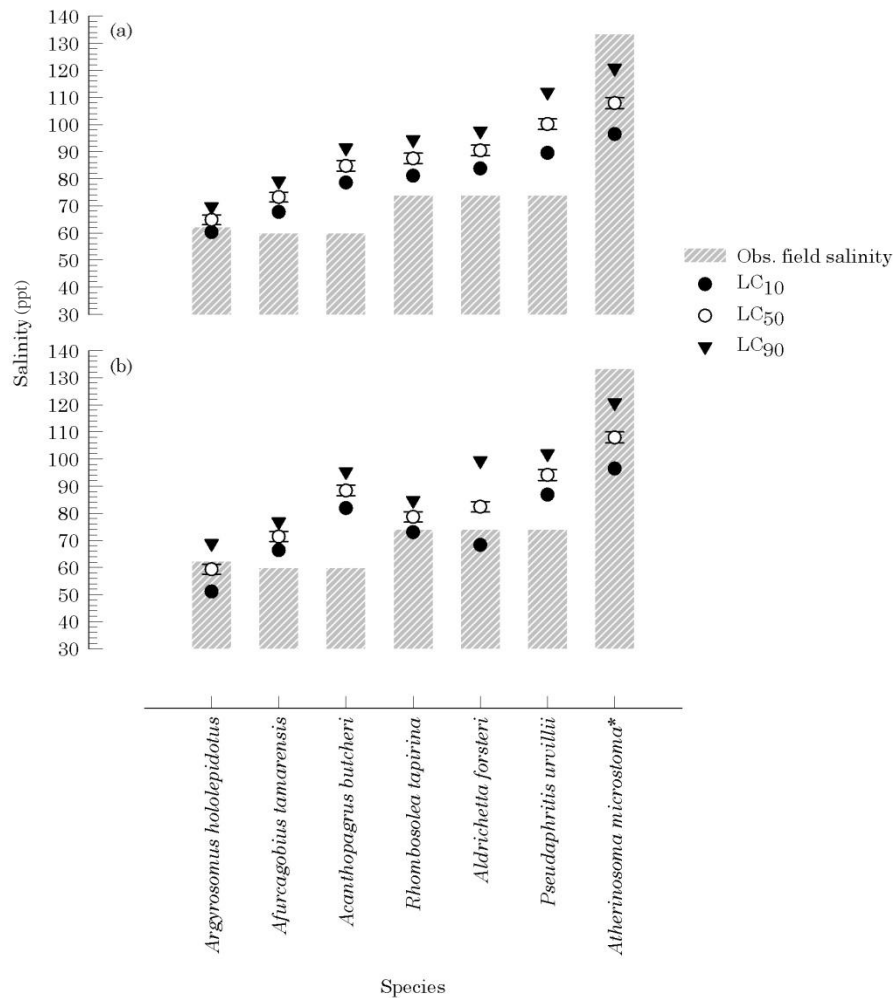
Figure 1. 10, 50 and 90 percent values ($LC_{10}$, $LC_{50}$ ($\pm$ standard error) and $LC_{90}$) for salinity determined at (a) 14°C and (b) 23°C for juveniles of six estuarine fish species, estimated using Probit analysis. * Lui 1969.

## Comparative vulnerability to hyper salinity impacts

Maximum salinities occupied by individual species from field observations by Noell *et al.* (2012) were compared to $LC_{50}$ values (determined in this study) for all species (Table 5) to validate the significance of LC estimates to conditions in the Coorong and Lower Lakes. The estimated relative order of vulnerability to increasing salinity across these species was identified; *A. hololepidotus* was considered to be the most vulnerable, whilst *P. urvillii* the most tolerant (although no trials were conducted on *A. microstoma* in this study).

**Table 5: Summary of LC$_{50}$ salinity estimates (expressed as ppt) for six species at 14 ºC and 23 ºC temperatures compared to field observations from Noell *et al.* (2009).**

| Vulnerability to high salinity | Common name | Species name | LC$_{50}$ | | Max. field obs. salinity |
|---|---|---|---|---|---|
| | | | 14 °C | 23 °C | |
| Most | Mulloway | *Argyrosomus hololepidotus* | 64.4 | 59.3 | 62.4 |
| | Tamar goby | *Afurcagobius tamarensis* | 73.2 | 71.4 | 60.1 |
| | Black bream | *Acanthopagrus butcheri* | 84.7 | 88.4 | 60.1 |
| | Greenback flounder | *Rhombosolea tapirina* | 87.5 | 78.6 | 74.1 |
| | Yellow eye mullet | *Aldrichetta forsteri* | 90.5 | 82.4 | 74.1 |
| | Congolli | *Pseudaphritis urvillii* | 100.1 | 94.1 | 74.1 |
| Least | Smallmouthed hardyhead# | *Atherinosoma microstoma* | 108 | | 133.5 |

[#] Lui (1969)

## Species-specific tolerance and distribution in relation to salinity

### *Mulloway (Argyrosomus hololepidotus)*

At 14 °C, *A. hololepidotus* mortalities occurred between 58 to 70 ppt (Figure 2), with an LC$_{50}$ estimate of 64.4 ppt (LC$_{10}$ = 60.3; LC$_{90}$ = 69.6) (Figure 1). At 23 °C mortalities occurred between 50 to 74 ppt (Figure 2) with an LC$_{50}$ estimate of 59.3 ppt (LC$_{10}$ = 51.1 ppt; LC$_{90}$ = 68.8 ppt) (Figure 12). Daily Feeding Response Score (DFRS) decreased at 52 to 54 ppt in both temperature treatments, before decreasing steadily with complete cessation of feeding at salinities of 62 ppt (14 °C) and 66 ppt (23 °C) (Figure 2). Stress Score (SS) was initially higher at 14 °C than at 23 °C; but rose in both treatments at 52 to 54 ppt with colouration darkening at 60 ppt and general inactivity occurring at 60 ppt (14 °C) and 64 ppt (23 °C) (Figure 2). The field distribution of *A. hololepidotus* was generally within 40 km of the Murray Mouth with occasional catches between March and September 2007 under salinity concentrations exceeding the 23 °C species LC$_{50}$ and approaching the 14 °C LC$_{10}$ (Figure 3).

Figure 2. Percent survival and feeding response and stress scores with increasing salinity at (a) 14°C ('winter') and (b) 23°C ('summer') temperatures for juvenile mulloway (*A. hololepidotus*).



Figure 3. Salinity concentration with distance from the Murray Mouth against time; with $LC_{50}$ values determined at 14°C and 23°C and field observations (○ = relative abundance, from Noell *et al.* (2012)) plotted for juvenile mulloway (*A. hololepidotus*).

11

### Tamar goby (*Afurcagobius tamarensis*)

At 14 °C mortalities occurred from 70 to 72 ppt (Figure 4), with an $LC_{50}$ estimate of 73.2 ppt ($LC_{10} =$ 67.7; $LC_{90} = 79.1$) (Figure 1). At 23 °C, mortalities occurred in salinities from 66 to 72 ppt (Figure 4) with an $LC_{50}$ estimate of 71.4 ppt ($LC_{10} = 66.3$; $LC_{90} = 76.8$) (Figure 1). DFRS decreased at 66 ppt at 14 °C, and 58 ppt at 23 °C, with this pattern continuing steadily until feeding ceased entirely in both treatments when ambient salinities reached 72 ppt (Figure 4). SS initially began to rise at 52 to 54 ppt in both temperatures, with change in colouration starting to occur at 58 ppt, followed by general inactivity at approximately 70 ppt in both treatments (Figure 4). The observed field distribution of *A. tamarensis* was generally within 20 km of the Murray Mouth with salinity conditions well within their tolerance range. Only during June 2007 did high salinity concentrations form close to *A. tamarensis* sites, however, the species was never caught under salinities approaching their estimated $LC_{50}$ (Figure 5).



Figure 4. Percent survival and feeding response and stress scores with increasing salinity at (a) 14°C ('winter') and (b) 23°C ('summer') temperatures for juvenile Tamar goby (*A. tamarensis*).

Figure 5. Salinity concentration with distance from the Murray Mouth against time; with $LC_{50}$ values determined at 14°C and 23°C and field observations (∘ = relative abundance, from Noell *et al.* (2012)) plotted for juvenile Tamar goby (*A. tamarensis*).

### Black bream (Acanthopagrus butcheri)

At 14 °C mortalities occurred at 80 to 86 ppt (Figure 6), with an $LC_{50}$ estimate of 84.7 ppt ($LC_{10}$ = 78.6; $LC_{90}$ = 91.4) (Figure 1). In contrast, at 23 °C mortalities occurred between 82 and 90 ppt, (Figure 6), with an $LC_{50}$ estimate of 88.4 ppt ($LC_{10}$ = 81.8; $LC_{90}$ = 95.3) (Figure 1). DFRS was observed to reduce initially between 76 and 78 ppt in both temperatures, and continued to decline until feeding ceased at 82 and 86 ppt at 14 °C and 23 °C, respectively (Figure 6). SS initially began to rise at 74 ppt in both temperatures, which included darkening colouration with fish generally becoming inactive at 74 ppt at 23 °C and 80 ppt at 14 °C (Figure 6). *A. butcheri* was generally collected within 20 km of the Murray Mouth and field salinities where the species was caught did not approach $LC_{50}$ estimates (Figure 7).
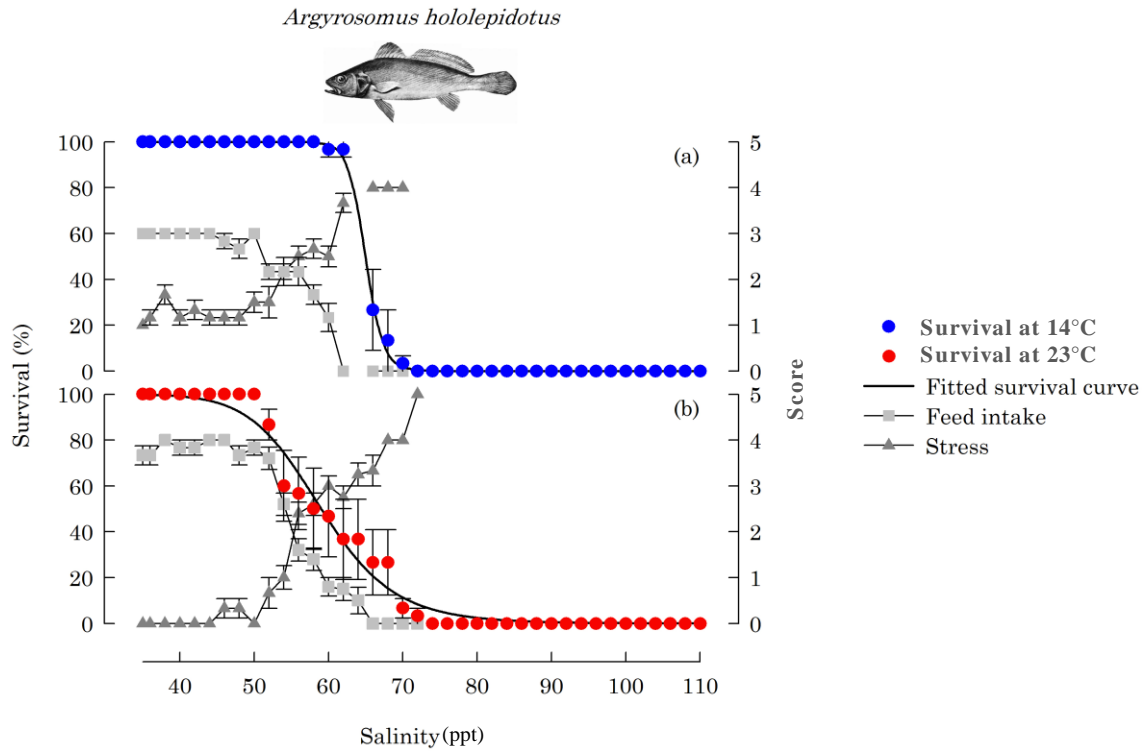
Figure 6. Percent survival and feeding response and stress scores with increasing salinity at (a) 14°C ('winter') and (b) 23°C ('summer') temperatures for juvenile black bream (*A. butcheri*).
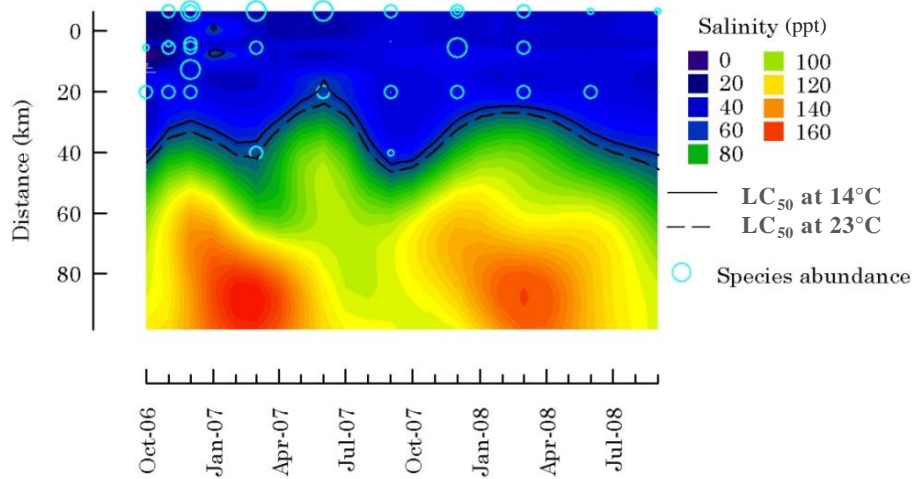


Figure 7. Salinity concentration with distance from the Murray Mouth against time; with LC50 values determined at14°C and 23°C and field observations (○ = relative abundance, from Noell *et al*., 2009) plotted for juvenile black bream (*A. butcheri*).

## *Greenback flounder (Rhombosolea tapirina)*

Mortalities occurred at 14 °C between 78 and 92 ppt (Figure 8) with an $LC_{50}$ estimate of 87.5 ppt ($LC_{10}$ = 81.1; $LC_{90}$ = 94.4) (Figure 1). In 23 °C, mortalities occurred between 68 and 82 ppt (Figure 8) with an $LC_{50}$ estimate of 78.6 ppt ($LC_{10}$ = 72.9; $LC_{90}$ = 84.7) (Figure 1). DFRS was observed to decline from 66 to 68 ppt in both temperature treatments, and continued to decrease until 82 ppt at 14 °C and 78 ppt at 23 °C (Figure 8). SS initially began to rise between 74 and 78 ppt in both temperatures, at which stage the colouration darkened and general inactivity became apparent in 76 ppt at 14 °C and 82 ppt at 23 °C (Figure 8). *R. tapirina* were frequently caught in reasonable numbers up to 50 km from the Murray Mouth. Whilst they were distributed close to saline waters in general, they were never caught in salinities matching or exceeding the $LC_{50}$ thresholds identified (Figure 9). They were, however, caught in salinities that exceed their $LC_{10}$ threshold (Figure 1), suggesting that some individuals of this species will enter and tolerate hyper-saline waters.
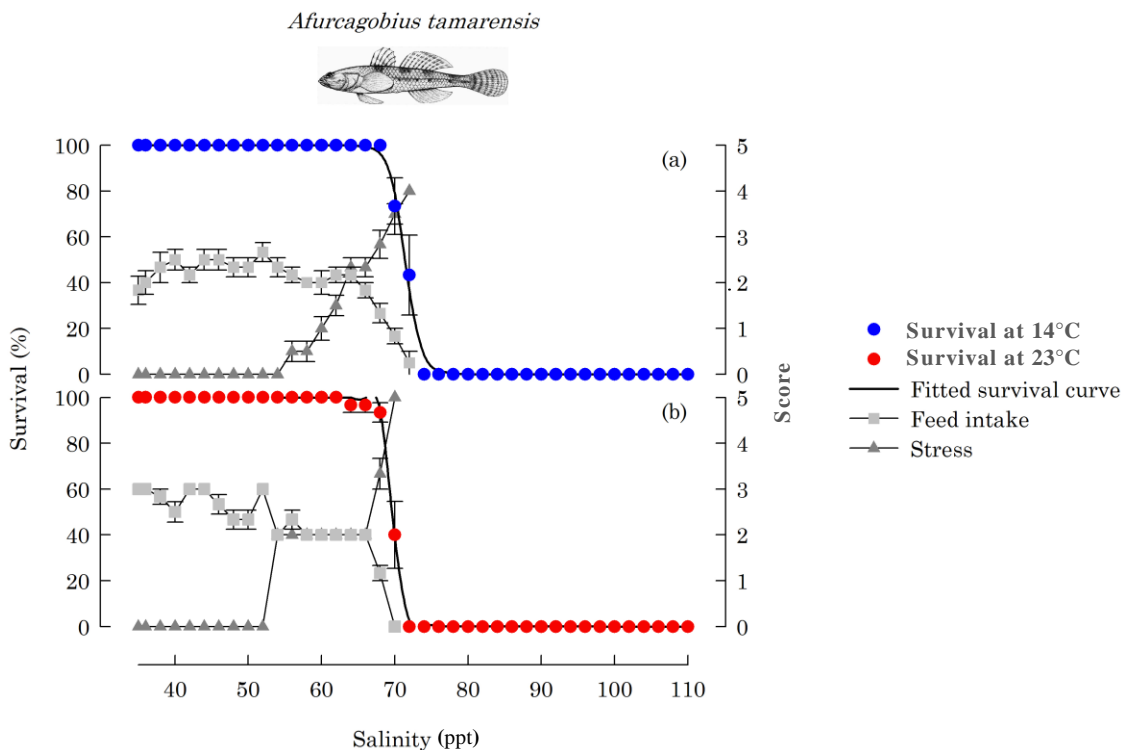


Figure 8. Percent survival and feeding response and stress scores with increasing salinity at (a) 14°C ('winter') and (b) 23°C ('summer') temperatures for juvenile greenback flounder (*R. tapirina*).
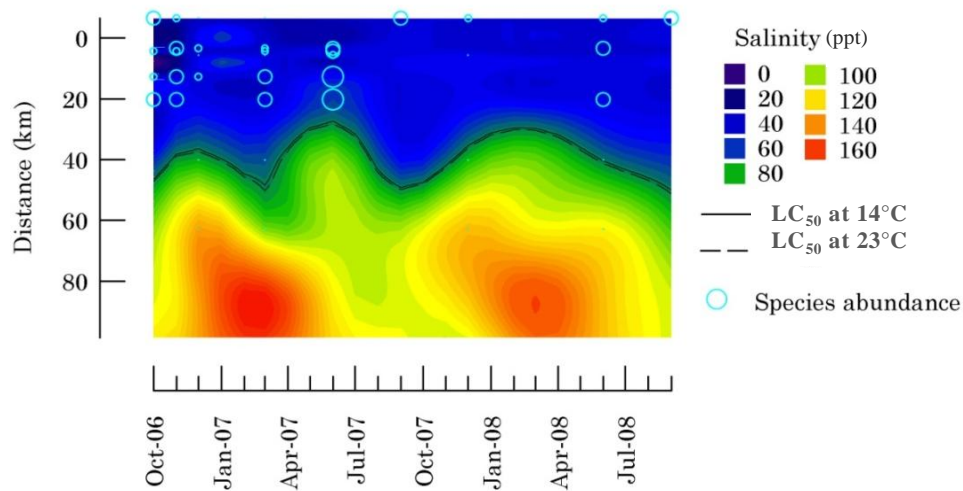
Figure 9. Salinity concentration with distance from the Murray Mouth against time; with $LC_{50}$ values determined at 14°C and 23°C and field observations (∘ = relative abundance, from Noell *et al.*, 2009) plotted for juvenile greenback flounder (*R. tapirina*).

### *Yellow eye mullet (Aldrichetta forsteri)*

At 14 °C mortalities occurred between 82 and 90 ppt (Figure 10) with an $LC_{50}$ estimate of 90.5 ppt ($LC_{10}$ = 83.8; $LC_{90}$ = 97.6) (Figure 1). At 23 °C, 20% of mortalities occurred between 50 and 70 ppt, with the majority occurring after 86 ppt (Figure 10) with an $LC_{50}$ estimate of 82.4 ppt ($LC_{10}$ = 68.3; $LC_{90}$ = 99.4) (Figure 1). At 14 °C DFRS started to decline from 84 ppt and continued to decline until feeding ceased completely at 88 ppt (Figure 10), whilst at 23 °C, FRS declined from 70 ppt and feeding did not cease until complete mortality at 90 ppt (Figure 10). SS initially began to increase with darkening colouration observed from 74 to 76 ppt at both temperatures and reduced activity was noticeable at 84 to 86 ppt (Figure 10). Erratic scoring for behavioural indicators suggests that this species may have had some baseline level of stress throughout the trials that may impact upon results, particularly under the 23 °C treatment. *A. forsteri* were recorded up to 40 km from the Murray Mouth. In June of 2008 this species was collected near to saline waters, but were never caught in salinities matching the $LC_{50}$ thresholds (Figure 11). They were, however, collected under salinities that could be lethal to at least 10% of the population (Figure 1).
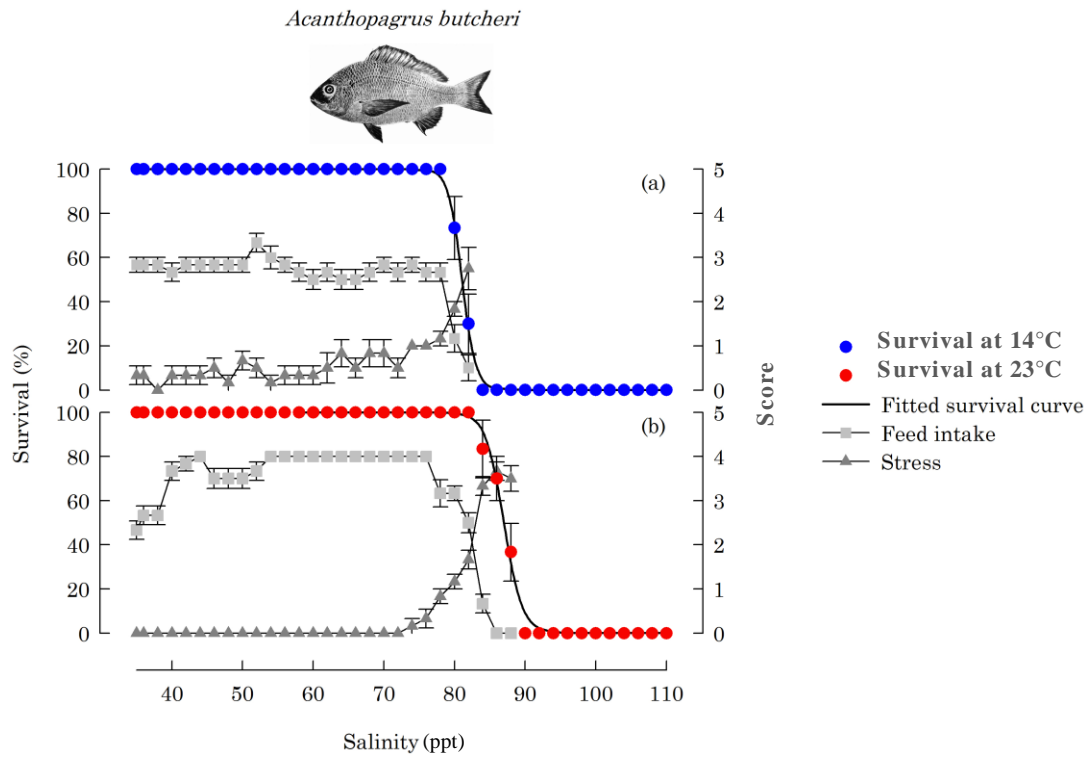
.
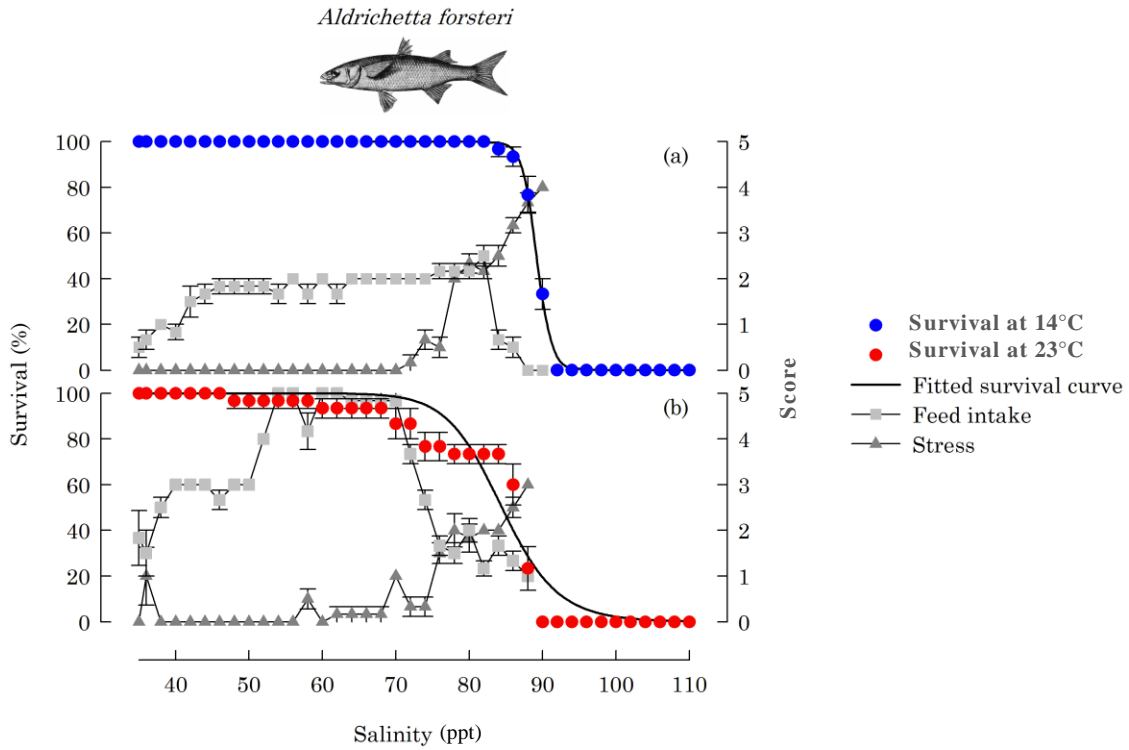
Figure 10. Percent survival and feeding response and stress scores with increasing salinity at (a) 14°C ('winter') and (b) 23°C ('summer') temperatures for juvenile yellow eye mullet (*A. forsteri*).
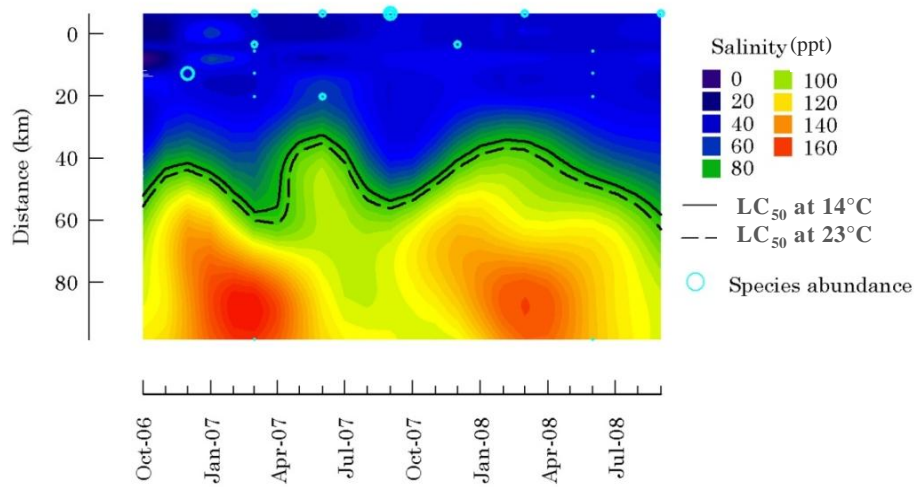


Figure 11. Salinity concentration with distance from the Murray Mouth against time; with $LC_{50}$ values determined at 14°C and 23°C and field observations ( ○ = relative abundance, from Noell *et al.*, 2009) plotted for juvenile yellow eye mullet (*A. forsteri*).

17

## *Congolli (Pseudaphritis urvillii)*

At 14 °C, mortalities occurred between 92 and 104 ppt (Figure 12) with an $LC_{50}$ estimate of 100.1 ppt ($LC_{10} = 89.5$; $LC_{90} = 111.9$) (Figure 1). At 23 °C, mortalities occurred between 84 and 98 ppt (Figure 12) with an $LC_{50}$ estimate of 94.1 ppt ($LC_{10} = 86.9$; $LC_{90} = 101.9$) (Figure 1). DFRS started to decrease at 82 ppt at 14 °C and 78 ppt at 23 °C, and continued to decrease steadily with exposure and fish ceased feeding at 88 ppt at both 14 °C and 23 °C (Figure 12). At 14 °C, SS began to rise at 68 ppt; darkening colouration was evident at 70 ppt (Figure 12). At 23 °C, SS began to increase at 74 ppt and darkened colouration was evident at 76 ppt (Figure 12). Reduced activity was noticeable after exposure to 88 ppt at both temperatures (Figure 12). The observed field distribution of *P. urvillii* was within 40 km of the Murray Mouth and largely within 20 km. Ambient salinity was in all cases well below the $LC_{50}$ estimates for this species (Figure 13).
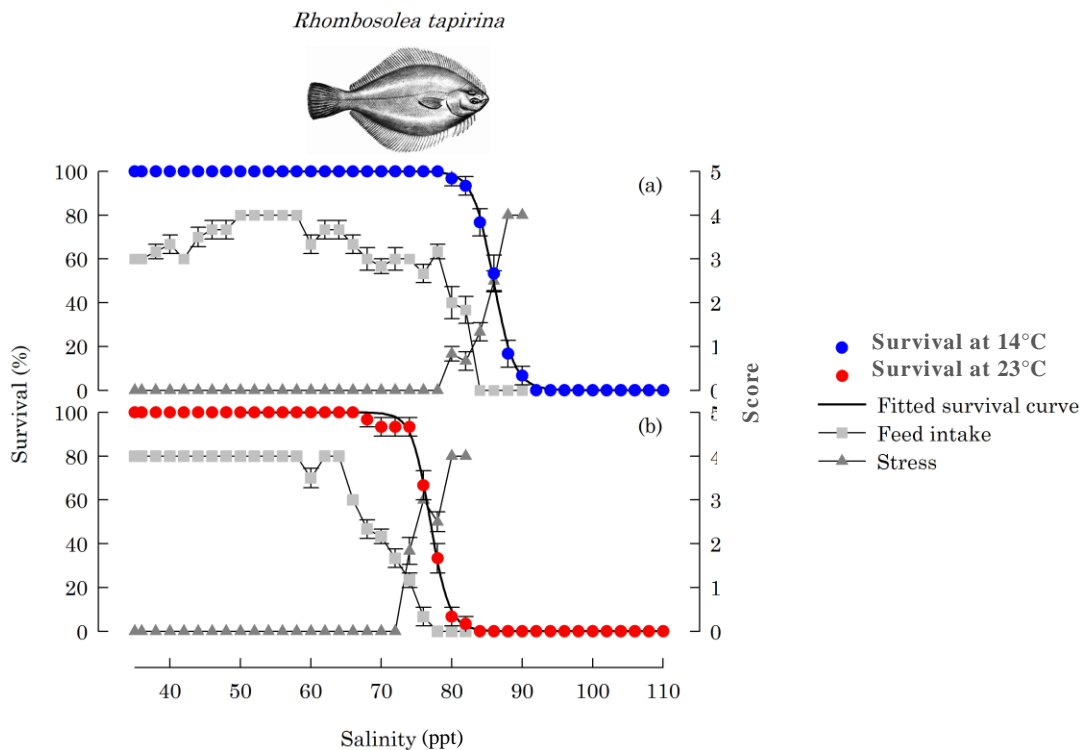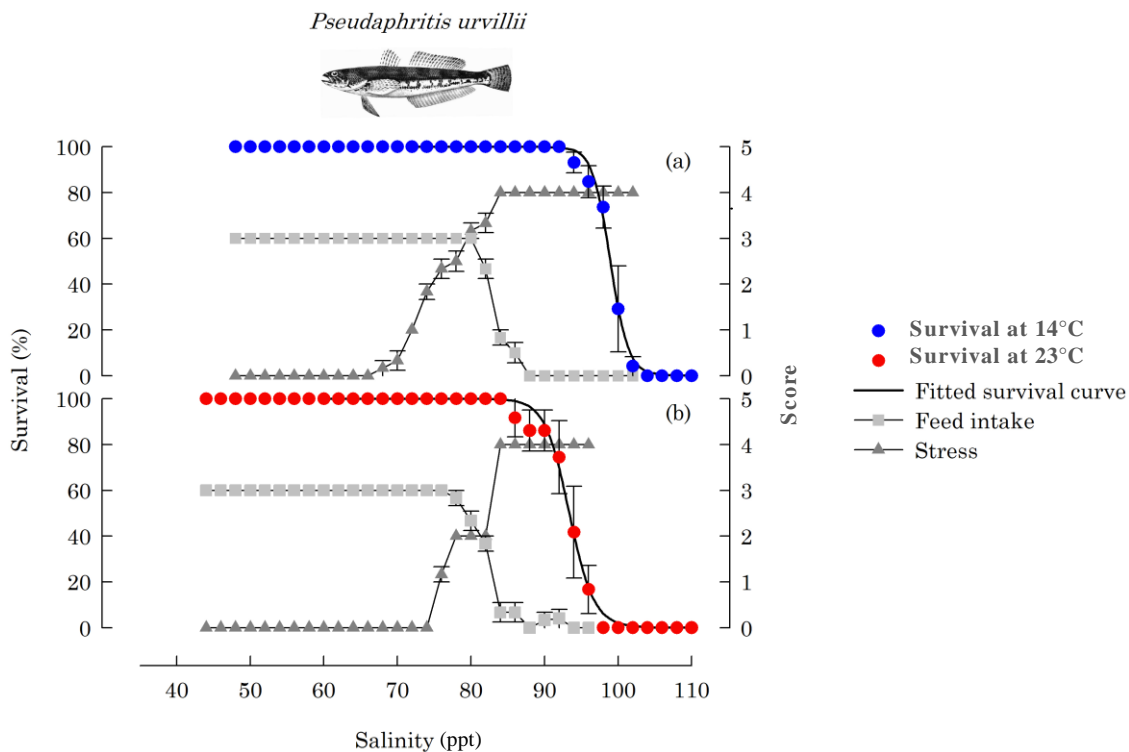


Figure 12. Percent survival and feeding response and stress scores with increasing salinity at (a) 14°C ('winter') and (b) 23°C ('summer') temperatures for juvenile congolli (*P. urvillii*).
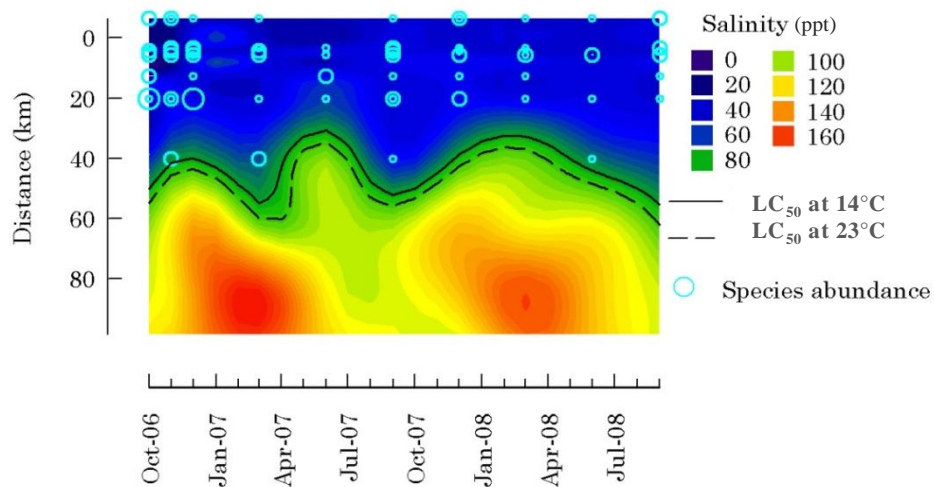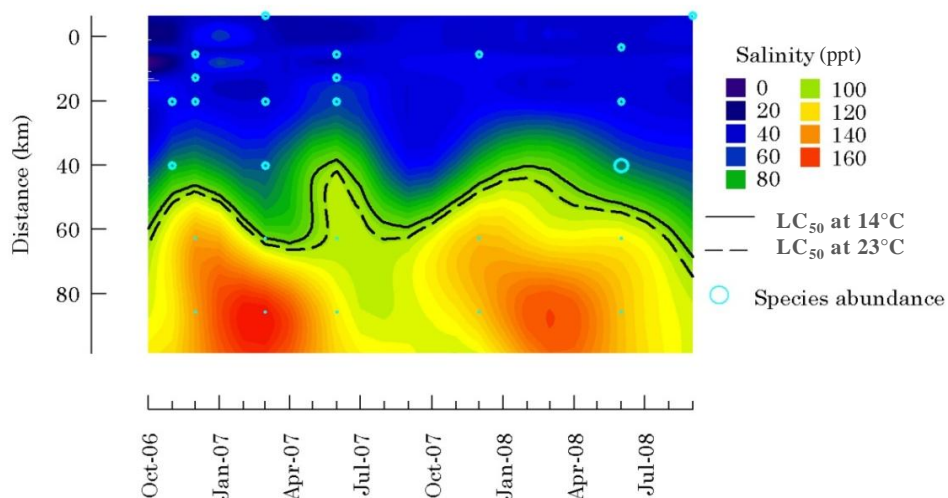
Figure 13. Salinity Concentration with distance from the Murray Mouth against time; with $LC_{50}$ values determined at 14°C and 23°C and field observations (∘ = relative abundance, from Noell *et al.*, 2009) plotted for juvenile congolli (*P. urvillii*).

### Smallmouthed hardyhead (*Atherinosoma microstoma*)

*A. microstoma* were collected up to 90 km from the Murray Mouth under field salinities greatly exceeding the $LC_{50}$ estimates identified by Lui (1969) using direct transfer methodologies (Figure 14). The highest abundances (larger circles) were recorded in salinities below the estimated $LC_{50}$, closer to the Murray Mouth; however, fish were recorded in ambient salinities up to 130 ppt (Figure 14) exceeding even the $LC_{90}$ threshold calculated for this species (Figure 1). The species was not captured in the hyper-saline waters present in some seasons away from the Murray Mouth (red areas).
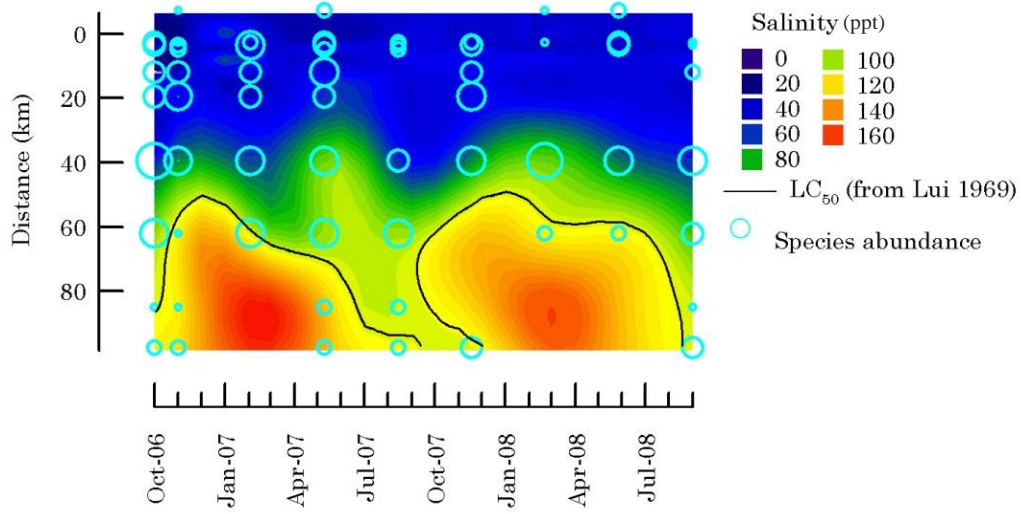
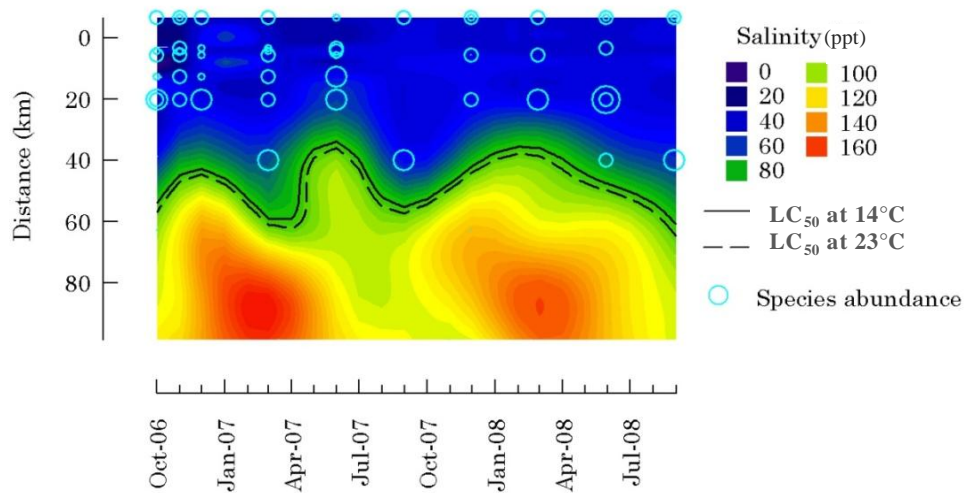Figure 14. Salinity Concentration with distance from the Murray Mouth against time; with $LC_{50}$ values determined by Lui (1969) and field observations ( ∘ = relative abundance, from Noell *et al.*, 2009) plotted for juvenile smallmouthed hardyhead (*A. microstoma*).

# 4. Discussion

## *Tolerance to hyper-saline conditions*

All fish species (juveniles) tested tolerated high salinity levels approximately twice the concentration of seawater. There was a gradient of relative tolerance across species with the highest tolerances recorded by *A. microstoma*, followed by *P. urvillii,* and *A. forsteri* which were tolerant of salinities greater than 80 ppt, regardless of water temperature. Although differentially affected by water temperature, *R. tapirina* and *A. butcheri* also recorded high tolerances (> 80 ppt). Slightly less tolerant, *A. tamarensis* and *A. hololepidotus* recorded tolerances between approximately 60 to 72 ppt. *A. butcheri* was the only species tested to record a higher tolerance value under the summer water temperature (23 °C) than the cooler winter temperature (14 °C). The maximum field salinities at which species were caught divided these species into two groups with *A. microstoma, P. urvillii, A. forsteri* and *R. tapirina* captured in salinities of over 74 ppt and *A. butcheri, A. tamarensis* and *A. hololepidotus* found only at salinities below 60 ppt. These field patterns suggest that the winter temperature tolerance thresholds for these species are more closely related to their distribution throughout the Coorong during 2006 to 2008.

## *Relating tolerance thresholds to ecological distributions*

A key outcome of the current study is the application of experimentally derived tolerance thresholds to the distribution of wild fish across a natural salinity gradient. The results suggest that threshold values, in particular $LC_{10}$ and $LC_{50}$, can approximate the maximum field distribution of species. In general, species tended to be caught in salinities far lower than threshold limits which concurs with results from Kefford *et al.* (2004). This could be viewed as an expected observation considering the range of chronic impacts that sub-lethal concentrations of salinity may have on the homeostasis of estuarine fish. Respiratory efficiency, energetic budgets, growth and immune responses are impacted at levels far below those directly lethal to individual fish (Geddes, 1979; Hart *et al.*, 1991; Haddy and Pankhurst, 2000; Boeuf and Payan, 2001; Akin and Neill, 2003).

By exception, two species were recorded from habitats more saline than their tolerance limits would suggest. *A. hololepidotus* recorded $LC_{10}$ and $LC_{50}$ thresholds at 23 °C that were lower than salinities at which they were recorded in the field. However, the catches of *A. hololepidotus* at these salinities only occurred during the cooler months and therefore the summer temperature threshold does not apply. Instead the winter (14 °C) threshold would apply and whilst salinity levels may still be high enough to restrict <10% of individuals, trials suggest most members of this species could survive under these salinity and temperature conditions.

Tolerance estimates were lower than habitat field salinities for *A. microstoma* and this was the only species not tested using gradual acclimation tolerance trials. Instead, threshold values were re-calculated from published data that used a direct transfer methodology. Direct transfer methods do not allow for any degree of acclimation to increasing salinities and can often under-estimate the ability of fish to physiologically adapt to rising salinity levels, leading to lower estimates of $LC_{50}$ (Ye *et al*., 2010). This result emphasises the importance of comprehensive and comparative assessments of species' tolerances when addressing the impacts of water quality extremes on fish health and survival, as the application of previously derived estimates may not allow valid comparisons across species.

If comparable methods were used for *A. microstoma*, as were used for other species in the current study, it is likely that LC estimates may more closely approximate field distribution limits. This would also render *A. microstoma* the most tolerant of all Coorong fish species as indicated by the field results, and it is hypothesised that the gradual acclimation derived $LC_{50}$ for this species is likely to approximate 140 ppt. This suggests that *A. microstoma*, is one of the most salt-tolerant fish species in the world alongside *Cyprinodon variegates* (Gunther, 1956).

### *Behavioural observations*

Behavioural response measures during gradual salinity increase provided additional insight into the sub-lethal salinity impacts. Increases in stress related behaviours and decreases in feeding and general activity always preceded or approximated the onset of mortalities during gradual salinity increase. These behavioural responses to gradual declines in water quality have proven extremely useful indicators of species' tolerance thresholds and allow the identification of pre-lethal thresholds at which fish are inclined to modify their behaviour to avoid excess stress or metabolic cost as conditions become impacting (McNeil and Closs, 2007). Long term trials also suggest that exposure over several months, even to salinity levels well below the lethal threshold of a species, can result in the ultimate death of over half of the treatment fish compared to controls. Under these conditions, behavioural measurements may identify impact levels undetectable through gross mortality measurements.

Furthermore, behavioural observations may reveal much about experimental conditions by providing indications of background stress. Trials for *A. forsteri,* for example, showed indications that fish may have been stressed and feeding abnormally during trials under the 23 °C treatment. This pattern coincided with low levels of mortality in experimental fish long before a general decline in survival was observed. The behavioural data therefore indicates that the result for *A. forsteri* might be more due to acclimation issue than test aquaria conditions. The failure of some individuals to acclimate was a likely cause of early mortalities rather than salinity impacts during the trial.

The restriction of almost all fish catches to areas of moderate salinity clearly supports the hypothesis that hyper-saline conditions in the Coorong are a primary environmental driver restricting the distribution of fish species to the fresher sections of the lagoon closer to the Murray Mouth. As the hyper-salinity mass contracts and expands seasonally, the distribution of many fish species can be seen to expand and contract also, generally bound by the confines of salinity tolerance limits. *A. hololepidotus, P. urvillii* and *R. tapirina* all moved away from the Murray Mouth during March and September 2007 when the hyper-saline zone contracted and were no longer caught at those sites once the zone expanded again towards the mouth. The most extreme case was *A. microstoma,* which is distributed almost ubiquitously outside of the constraints of their probable salinity threshold, expanding and contracting with the limits of the hyper-saline mass.

### *Ecological management and significance*

These results have implications for the provision of freshwater inputs into the Coorong and Lower River Murray. They indicate that freshwater inflows of subsequent size and duration to push back or break down the hyper-saline mass will most likely result in the expansion of key marine and estuarine species southward into the Coorong lagoon, expanding from remaining source populations that persist within marine environments close to the Murray Mouth. Furthermore, the data suggests that salinity levels do not need to be significantly fresh and that resumption of marine conditions is likely to facilitate the expansion of most fish species back into the lagoon, assuming that ecological fidelity also recovers and the ecological processes necessary to support fish populations are also able to recover from hyper-saline conditions.

The distributional data also shows that the true estuarine species, *A. butcheri* and *A. tamarensis*, are perhaps the least likely to respond quickly to improvements in saline conditions due to their apparent reluctance to move toward a hyper-saline mass, even during seasonal retreats southward. It is likely that these species are persisting within sub-optimum marine environments around the Murray Mouth area and therefore are likely to be constrained by the hyper-saline mass and more impacted by standard salinity levels (Potter and Hyndes, 1994). These species have relatively lower tolerances and are therefore likely to be most impacted by any further expansion of the hyper-saline mass into the current area of fish abundance near the Murray Mouth. Whilst also relatively intolerant, *A. hololepidotus* is also highly mobile and therefore able to retreat rapidly to marine environments to which they are well adapted.

# 5. References

Akin S. and Neill W. H. (2003). Routine metabolism of mosquitofish (*Gambusia affinis*) at three different salinities. *Texas Journal of Science* **55(3)**: 255-262.

Blaber S. J. M., Brewer D. T. and Salini J. P. (1989). Species composition and biomasses of fishes in different habitats of a tropical Northern Australian estuary: Their occurrence in the adjoining sea and estuarine dependence. *Estuarine, Coastal and Shelf Science* **29(6)**: 509-531. Boeuf G. and Payan P. (2001). How should salinity influence fish growth? *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* **130(4)** 411-423. Boon P. I. (2000). Biological impacts of changes to water level and salinity in the Coorong. Report to the Upper South East Dryland Salinity and Flood Management scheme and Victoria University, Melbourne.

Clunie P., Ryan T., James K. and Cant B. (2002). Implications for rivers from salinity hazards: scoping study. Department of Natural Resources and Environment, Murray Darling Basin Commission, Strategic Investigations and Riverine Program No. Project R2003, Melbourne, Australia.

Flemer D. A. and Champ M. A. (2006). What is the future fate of estuaries given nutrient over-enrichment, freshwater diversion and low flows? *Marine Pollution Bulletin* **52(3)** 247-258. Geddes M. (1987). Changes in salinity and in the distribution of macrophytes, macrobenthos and fish in the Coorong Lagoons, South Australia, following a period of River Murray flow. Transactions of the Royal Society of South Australia **111**: 173-181.

Geddes M. (2005a). The ecological health of the North and South Lagoons of the Coorong in July 2004. Final report. SARDI Aquatic Sciences, No. RD03/0272-2, Adelaide.

Geddes M. (2005b). Ecological outcomes for the Murray Mouth and Coorong from the managed barrage release of September-October 2003. Final Report. SARDI Aquatic Sciences, No. RD/03/0199-2, Adelaide.

Geddes M. and Butler A. (1984). Physicochemical and biological studies on the Coorong lagoons, South Australia, and the effect of salinity on the distribution of the macrobenthos. Transactions of the Royal Society of South Australia **108(1)**: 51-62.

Geddes M. C. (1979). Salinity tolerance and osmotic behaviour of European carp (*Cyprinus carpio* L.) from the River Murray, Australia. Transactions of the Royal Society of South Australia **103(7)**: 185-189.

Gunther, G. (1956). Some relations of faunal distributions to salinity in estuarine waters. *Ecology* **37**: 616-619.

Haddy J. and Pankhurst N. (2000). The effects of salinity on reproduction development, plasma steroid levels, fertilisation and egg survival in black bream *Acanthopagrus butcheri*. *Aquaculture* **188**: 115-131.

Hart B. T., Bailey P., Edwards R., Hortle K., James K., McMahon A., Meredith C. and Swadling K. (1991). A review of the salt sensitivity of the Australian freshwater biota. *Hydrobiologia* **210**: 105-144.

Hildebrand M. and Goslow G. (2001). 'Analysis of Vertebrate Structure, 5th Edition'. John Wiley & Sons, New York.

James K., Cant B. and Ryan T. (2003). Responses of freshwater biota to rising salinity levels and implications for saline water management: a review. *Australian Journal of Botany* **51**: 703-713.

Kefford B. J., Papas P. J., Metzeling L. and Nugegoda D. (2004). Do laboratory salinity tolerances of freshwater animals correspond with their field salinity? *Environmental Pollution* **129**: 355-362.

Lui L. (1969). Salinity tolerance and osmoregulation of *Taeniomembers microstomus* (Gunther, 1861) (Pisces: Mugiliformes: Atherinidae) from Australian salt lakes. *Marine and Freshwater Research* **20(2)**: 157-162.

McNeil D. G. and Closs G. P. (2007). Behavioural responses of a south-east Australian floodplain fish community to gradual hypoxia. *Freshwater Biology* **52**: 412–420.

Montagna P. A., Alber M., Doering P., and Connor M. S. (2002). Freshwater Inflow: Science, Policy, Management. *Estuaries* **25(6b)**: 1243–1245.

Noell C. J., Ye Q., Short D. A., Bucater L. B. and Wellman N. R. (2009). Fish assemblages of the Murray Mouth and Coorong region, South Australia, during an extended drought period. CSIRO: Water for a healthy Country National Research Flagship and South Australia Research and Development Institute (Aquatic Sciences), Adelaide.

Pollard D. A. (1971). The biology of a landlocked form of the normally catadromous salmoniform fish *Galaxias maculatus* (Jenyns). *Australian Journal of Marine and Freshwater Research* **22**: 91-123.

Potter I. C., Beckley L. E., Whitfield A. K. and Lenanton R. C. J. (1990). Comparisons between the roles played by estuaries in the life cycles of fishes in temperate Western Australia and Southern Africa. *Environmental Biology of Fishes* **28**(1),143-178.

Potter I. C. and Hyndes G. A. (1994). Composition of fish fauna of a permanently open estuary on the southern coast of Australia, and comparisons with nearby seasonally closed estuary. *Marine Biology* **121**: 199-209.

Pressey R. L. and Adam P. (1995). A review of wetland inventory and classification in Australia. *Plant Ecology* **118**(1),81-101.

Vega-Cendejas M. E. and Hernández de Santillana M. (2004). Fish community structure and dynamics in a coastal hypersaline lagoon: Rio Lagartos, Yucatan, Mexico. *Estuarine, Coastal and Shelf Science* **60(2)** 285-299. Vorwerk P. D., Froneman W. P., Paterson A. W., Strydom N. A. and Whitfield A. K. (2008). Biological responses to a resumption in river flow in a freshwater deprived, permanently open Southern African estuary. *Water SA* **34(5)**: 597-604.

Walker D. J. (2002). The Behaviour and Future of the River Murray Mouth. Centre for Applied Modelling in Water Engineering, Department of Civil and Environmental Engineering, Adelaide University.

Webster I. T. (2005). An overview of the hydrodynamics of the Coorong and Murray Mouth: water levels and salinity - key ecological drivers. Water for a Healthy Country National Research Flagship CSIRO Report Series Technical Report No. #/2005.

Wedderburn S. D., Walker K. F. and Zampatti B. P. (2008). Salinity may cause fragmentation of hardyhead (Teleostei: Atherinidae) populations in the River Murray, Australia. *Marine and Freshwater Research* **59(3)**: 254-258.

Whitfield A. K. (1999). Ichthyofaunal assemblages in estuaries: a South African case study. *Reviews in Fish Biology and Fisheries* **9**: 151-186.

Whitfield A. K. (2005). Fishes and freshwater in southern African estuaries - A review. *Aquatic Living Resources* **18**: 275-289.

Ye, Q. Cheshire, K. M. J. and McNeil, D. G. (Editors) (2010). Influences of salinity, water quality and hydrology on early life stages of fishes in the Lower River Murray, South Australia.' South Australian Research and Development Institute (Aquatic Sciences) SARDI Publication No. F2009/000470-4. SARDI Research Report Series No. 446: Adelaide.

Zampatti B. P., Bice C. M. and Jennings P. R. (2010). Temporal variability in fish assemblage structure and recruitment in a freshwater-deprived estuary: The Coorong, Australia. *Marine and Freshwater Research* **61**(11),1298-1312.